

2
NBRL 48th TPR, April 1973

RELEASE OF BACTERIAL SPORES FROM THE INNER WALLS OF A
STAINLESS STEEL CUP SUBJECTED TO THERMAL STRESSES
AND MECHANICAL SHOCK

H. Wolochow, M. Chatigny and J. Hebert

The release and fallout of particulates from surfaces afforded thermal or impact stress is of concern to workers responsible for control of contamination of Mars from planetary landing vehicles. In this study we have used a metal vessel contaminated by aerosols of spores as a model system and have examined the fallout of spores as affected by various mechanisms. Thermal stresses simulating those expected on the Mars lander dislodged approximately .01% of the aerosol deposited surface burden as did a "landing" shock of 8 to 10G deceleration. Spores imprinted by finger or swab contact yielded similar results. In all cases where repeated cycling of temperature, motion, or shock were employed the majority of fallout occurred in the first cycle. Particles released from the surface were predominantly in the size range 1 to 5µm.

One of the major experiments in the planetary exploration program will be the soft landing of a spacecraft on the planet Mars. Included in the lander module will be various pieces of equipment designed to assess the presence of living entities (microbial cells) on this planet. Experimental equipment will be contained in metal vessels. Although it is planned to use thermal processed terminal sterilization of the spacecraft and a high degree of assurance of sterility will be attached to the entire process, there still remains a chance for viable microbial cells to be residual on inner surfaces on the containment vessels. If cells are dislodged during spacecraft landing, then residence on Mars would be a threat to the planetary quarantine and the integrity of the life-detection system. Stresses which might encourage dislodgement of cells deposited on surfaces include minute changes in vessel dimensions resulting from thermal gradients and deceleration distortion from landing shock. Furthermore, dimensional changes of the container (opened on Mars) would induce atmosphere flows through small openings; these flows could carry with them cells rendered airborne during surface deformation. The ease with which cells could be dislodged may well be a function of how they were deposited initially.

The experiments to be described were undertaken to investigate the potential for dislodgement of microbial spores deposited on stain-

N73-30987

NASA-CR-133868) RELEASE OF BACTERIAL
SPORES FROM THE INNER WALLS OF A
STAINLESS STEEL CUP SUBJECTED TO THERMAL
STRESSES AND (Naval Biomedical Research
Lab., Oakland) 24 p HC \$3.25 CSCL 06M

Unclas

13549

G3/04

Naval Biomedical Res. Lab.

NASA

W-13450

less steel surfaces subjected to thermal and deceleration stresses. The stresses were those derived from preliminary data on Mars surface conditions and planned soft landing deceleration (ca. 125° C temp. variation diurnally, 8 to 12g deceleration). The data presented herein do not consider the aerosol fall rate effects of Martian gravity and "thin" atmosphere, nor do they consider the thermal insulation of the enclosed spaces.

Bacterial spores, deposited from aerosols, were dislodged by thermal stresses. The "yield" was less than $10^{-2}\%$ of the burden. Deceleration of 8-12 g dislodged 0.6×10^{-2} to $3 \times 10^{-2}\%$ of aerosol deposited spores. Spores "imprinted" on surfaces by swabs or coated finger were dislodged in 2×10^{-2} to 1×10^{-2} of spore burden by thermal stress.

MATERIALS AND METHODS

1. Test vessel. Fig. 1 is a dimensional sketch of the test fixture. The vessel is made of type 304 stainless, helium arc welded, with smooth inner joints and a tapered interior with no re-entrant surfaces. Fluids or gases can be circulated through the jacket.

A metal band on which a rod ($\frac{1}{2}$ " dia.) is welded at right angles was clamped around the test vessel. The test vessel can be held on a ring stand in the desired orientation.

In order to maximize collection of particles dislodged from the test vessel, a circular metal disk was supported on another disk with friction fingers which gripped the test vessel on its outer surface near the open end. A gap of about $1/8$ " existed between plate and vessel. A one-holed rubber stopper was inserted into centrally located holes through these plates.

In some experiments designed to simulate "thermal breathing" output from larger volume vessels, a small bore metal tube was inserted into the vessel so that tube end was about 2-3 cm from the closed end of the vessel. Sterile dry air (in-line filter, Pall Mfg. Co.) was blown into the test vessel at various volumetric rates measured by a rotameter. This arrangement was not used in all experiments.

Two other versions of this disk system were used; these will be mentioned below.

2. "Contamination" of test vessel.

a. An aerosol of washed spores of Bacillus subtilis var niger (BG spores) was generated for 10 min by a Chicago-type (two-fluid

non-refluxing) atomizer (ca. 0.75 cfm), fed by a motorized syringe pump which delivered ca. 0.75 ml per min of an absolute alcohol suspension (ca. 3×10^{10} ml) of washed lyophilized spores. A suspension of this concentration sprayed from this type atomizer will be produced with a nominally log-normal particle size distribution with the number mean diameter approximately 5-8 μ . Each particle will contain from 1 to 5 spores. The aerosol was directed into a length of sheet metal pipe, with about 1.15 cfm secondary air drawn through by a vacuum source at the distal end of the tube. The secondary air was supplied from laboratory dry air mains (ca. -30 C dewpoint) redried through tandem activated alumina beds (Pittsburgh "Lectrodryer"). The test vessel was held on a "T" joint, some 50 cm from the aerosol inlet, in such a manner that the inside of the vessel faced up, normal to the air flow (Fig. 2). The entire apparatus was held in a large tank (Fig. 3) held at a moisture level of ca. 50-100 ppm H₂O. Manipulations were carried out through rubber gloves. After the inside of the vessel was "contaminated", a gasketed lid was placed over its opening (and held by rubber bands) prior to removal to a "dry box", which served as a test chamber, in which subsequent manipulations and sampling were carried out. A thin film of silicone grease was spread on the rim of the opening of the test vessel. The outside of the "contaminated" test vessel was decontaminated with dilute (25 to 100 ppm) hypochlorite solution and air-dried prior to removal to the dry box.

b. Contamination by swab or fingers. Washed suspensions of spores were swabbed onto inner surfaces with a cotton-tipped stick. In another trial, tips of fingers were dipped in this suspension and imprints made on several locations within the vessel. In both cases, the test vessel was equilibrated with the atmosphere inside the dry box prior to subsequent thermal cycling.

3. Dry Box. A section of a Class III hood system was used (Fig. 4). This section, about 30 in. long, had gasketed doors on either end and dual glove ports on the front. The box was flushed with air which was dried by an Electrodrrier, until the water vapor level was below 100 ppm. Penetrations into the box were available for supply of flushing air, vacuum and other services.

Between trials the inside of this box was decontaminated by atomizing formalin. After an air-wash, ammonium hydroxide solution was atomized for neutralization of deposited paraformaldehyde and air-wash continued.

4. Assay of Bacillus spores. Washed spores of Bacillus subtilis var niger were used throughout. Counting of viable spores was done on Trypticase Soy Agar (BBL) (TSA). This medium was used in all the collections. Plates were incubated at 37 C for 24 hr prior to counting. Distilled water was used for dilution purposes.

a. Aerosols

i) All glass impingers (AGI-30) sampling at 12.5 l/min were used, operated with short sections of tubing intruding into the dry box. The collecting fluid, water, was assayed for spores.

ii) Setting plates. Sterile, 100 mm petri dishes were placed on the bottom of the dry box (Fig. 5). Just before a trial, the lids were removed. After the desired time interval, the lids were replaced, the dishes removed and 20-25 ml molten (50-60C) TSA was added.

Settling plates containing TSA were used in several early experiments, where low humidity was not a requirement.

iii) Slit samplers. One cfm air was sampled onto TSA plates which rotated at 2 rpH. By noting starting location, time of arrival of spores could be determined as a function of manipulations.

iv) Andersen sampler. A six-stage unit was used to estimate both colony forming unit (CFU) concentration and particle size distribution. Unit operated at 1 cfm.

v) Stainless steel disc. For spore assay, plates were immersed in distilled water and sonicated for 10 min. Aliquots were filtered through a 0.45 μ m membrane filter, which was placed on freshly poured TSA.

vi) Gelman glass fiber filter discs (13 mm). These discs were blended (Waring) in 100 ml H₂O for 3 min. Suspension filtered through 0.45 μ m pore MF, which was treated as in (v) above.

b. Total surface burden. The test vessel was filled (350 ml) with sterile water. The probe of a Branson sonifier was immersed and power set at 100 watts. Immersed depth was adjusted for optimal action for 1.0 minute of sonication. Viable counts of spores were made as above.

5. Measurement of water vapor. A Beckman electrolytic hygrometer (Model 179) was used. Moisture contents are expressed as parts per million water vapor (w/v).

6. Thermal cycling of test vessel.

a. A temperature above ambient. A steam generator (resistance wire in water contained in a 2 l heavy wall filter flask, 20-30 volts) external to the dry box, delivered steam at essentially atmospheric pressure to the test vessel jacket, the return tubing from which was external to the dry box. A metal thermometer was placed in an unused inlet to the test vessel. Alternatively, a 24 ga iron-constantin thermocouple was taped to the vessel wall and a 0-10 mv digital voltmeter (Digitec) was used for EMF measurement, with a water-ice mixture containing the cold junction. Warm water, generated by passage of tap water through a section of pipe containing an electric heater, was also used in some trials.

b. Temperature below ambient.

i) Ice water circulated through test vessel jacket by a small pump.

ii) Ethylcellusolve, cooled by dry-ice was circulated as above.

iii) Liquid nitrogen was pulled into the test vessel by a vacuum line connected to one of the openings into the jacket of the vessel.

7. Deceleration. Dropping the test vessel from a height of about 25 cm onto a 3 cm thick pad of sponge gave about 10-12 g deceleration force as measured by a calibrated accelerometer. Dropping approximately 12 in. onto a metal surface yielded measured deceleration forces in excess of 100 g. The spores dislodged were caught on a metal plate (no central hole) covering the open end of the test vessel.

RESULTS

1. Spore burden in test vessel. Deposition from aerosol gave 1 to 30×10^7 spores, as did cotton swab. Approximately 2.7×10^8 spores were recovered following "inoculation" by contaminated fingers (Table 1).

2. Dislodgement of aerosol-deposited spores.

a. Air flow alone (spores recovered on petri plates). In 2 trials, air flow into the vessel (opening down) was changed from 0 to 85 l/min (Table 2). At the highest flow, over 1000 CFU were collected directly below the test vessel; at 10 l/min or less the numbers

were somewhat smaller, and did not parallel air flow change. At the highest flow rate, it is probable that many large particles were dislodged (note higher central plate count) and that the small particles were rendered airborne and did not reach the settling plates in the short sampling period used.

At 24 C, over a 1 hr period, a total of 54 CFU were recovered with no air flowing. When air at 150 ml/min was admitted, over a 20 hr period, 70 CFU were recovered (Table 3). Thus, it is concluded that a small air flow was found to be ineffective, of itself, in dislodging particles.

b. Thermal shifts.

i) With no air flow, no measurable effect on deposited BG spores was found when test vessel, laden with 2.6×10^8 spores was held for 2 hrs at 100 C (Table 4). During this time, a total of 159 CFU were recovered on settling plates below and near the test unit.

During a 2 hr period, at approximately 24 C, 43 CFU were recovered on 12 settling plates after putting the test unit in place in the dry box. Without disturbing the vessel, the temperature was cycled 4 times between 4 C and 100 C over a 2 hr period. A total of 26 CFU was recovered on 12 settling plates (Table 5). Similar results were obtained when temperature was shifted from 24 C to 95 C and then down to 5 C (Table 6). In another trial in a 2 hr "pre-thermal period", 18 CFU were recovered on 8 plates. When temperature was raised to 100 C, lowered to 2 C and raised to 24 C, 91 CFU were recovered on 11 plates, 62 being found on the plate immediately below the vessel (Table 7).

ii) Spores recovered by means of the metal disc. It was recognized that the distributed plates did not collect all particles released from the unit and the cover plate and disc were added. With the vessel in the vertical position (opening down) temperature was cycled as shown in Fig 6. Number of CFU caught during each cycle and in the 23 hrs between cycles 2 and 3 is also shown. It is evident that heat-treatment released spores, which fell on the sampling plate; building vibration probably accounted for spores released at almost the same rate over the 23 hr period between cycles 2 and 3.

Placing the vessel in the horizontal position yielded data as shown in Fig. 7. All attempts were made to minimize mechanical dislodgement during sample disc placement. It would seem that even under this orientation of the unit, spores were caught on the sample disc, although in numbers smaller than in the previous trial. It is also noted that holding the test unit for 17 hours in this position yielded 80 CFU (about 3x the output from the second cycle).

iii) Collection of spores on metal disc and from aerosol by slit sampler and AGI-30. One thermal cycle, with vessel vertical, was carried out. Temperatures ranging from room to -65°C to 100°C and back to room. The profile of aerosol concentration vs. time is shown in Fig. 8. Virtually all the spores, as aerosol were collected during the first cooling from room temperature and then heating to 100°C phase. Very few were recovered during cooling from 100°C to room temperature. Most spores were dislodged during the first cooling phase, indicating that the first dimensional change of the vessel is of primary importance in particle dislodgement.

The slit sampler collected 33 CFU per liter of air, while the AGI-30 collected 42/l. Thus, there were about 1.3 spores per particle, since the slit device enumerates particles (regardless of spore numbers per particle) while the count from the AGI-30 more nearly represents individual spores. The test disc had 604 colonies on it.

In a somewhat similar experiment, 3 temperature cycles were carried out, ranging from ca. 70°C to 40°C, over a 90 min period (Fig. 9). With a further 18 hr period at 22°C, 1 more temperature cycle was imposed (liquid N₂, to 85°C, to 22°C). Aerosol concentration ratios were measured with a slit sampler and fallout on a petri dish immediately below the vessel. In one instance an Andersen sampler was used. CFU were recovered in small numbers (less than 30) on all stages. Most of the spores were released during the initial cooling phase, both as aerosol and as fallout particles. The number of spores, obtained in the fourth cycle, was greater than in the previous 3, where the temperature gradients were smaller. During the last cooling cycle, the temperature was reduced to the point where liquid oxygen droplets collected on the surface of the vessel and ran down, onto the fallout plate, where they evaporated. It is quite likely that these droplets carried with them the spores which appeared in the fallout plates and in the aerosol. With the test vessel open and up at a temperature of -196°C (LN₂) ca. 5 ml of liquid O₂ collected over 15 min. This liquid evaporated rapidly when the LN₂ supply was closed off.

The test vessel, held in the horizontal position, was fitted with the metal collection plate which supported an air inlet at 125 ml/min (0.3 volumes/min) and an outlet (Figs. 10, 11). The latter was connected to an in-line filter holder which held a 13 mm glass fiber filter pad (Gelman). This filter was assayed as described above. Two trials were carried out, with 3 temperature cycles each (ca. 40 min. per cycle (Fig. 12, 13)). From the results in Table 8, it can be seen that spores were released onto the collection plate and as aerosol. From ca. 10⁻²% to 10⁻⁴% of the total burden were dislodged, and aerosol concentration ranged from 6 to 350 CFU/l of air.

Deceleration. The test vessel (spore burden deposited from aerosol) with metal collection plate over the open end was dropped (open end down) about 30.5 cm onto a polyurethane pad (acc = 8 to 12 G). In 4 trials, the fraction of the total burden recovered on the plate varied from 0.6×10^{-4} to 2.8×10^{-4} (Table 9). When test vessel was subjected to deceleration of over 100 G, a maximum of 3.9% of the spore burden (aerosol deposited) was recovered on the test disc (Table 10).

Release of spores deposited by swab or fingerprint. These modes of contamination deposited spores which were released in the same order of magnitude as when spores were deposited from an aerosol (Table 11).

DISCUSSION

In limited-scope trials as are described in this report, it is difficult to compute significance of the data and it is required that results be clear-cut and without great reservations concerning the test method. With a few exceptions, these tests showed an increased (compared to quiescent) rate of fallout of particles from the test unit when it was thermally cycled or afforded physical shock. However, the simple act of handling or even holding the test unit also dislodged particles indicating that the particles were not very firmly attached. A scanning electron-microscope photo of a spore particle (Fig. 14) deposited on a stainless steel slide in this system showed that the particles had little material around them. It is likely that the test conditions provided near "worst case" as far as secure attachment of the particles was concerned.

The aerosol depositing system used provides a "log-normal" particle size distribution under normal conditions. From the results, it appears that these sprays had many particles in sizes below 5μ . These indeed appeared to be responsible for most of the fallout. This is shown by the ratio of AGI-30 to slit sampler counts (ca. 1.33) and by Andersen sampler counts. This particle size distribution is fortuitously, probably a good simulation of that occurring on "cleaned" surfaces.

The thermal deformation of the test vessel provided a rather small dimensional change which is on the order of 1.3×10^{-3} cm/cm linearly for a 100° C change in the temperature range used. It is not likely that this would provide simple shear forces exceeding the particle to surface adhesion forces but that a more complex relationship exists. In at least one test where LN_2 was used for coolant, the precipitation of liquid O_2 on the test surface provided a very large increase in particle removal.

The question of thermal "pumping" of the atmosphere in an enclosure is not clearly resolved by these experiments. However, the data of Table 8 do show that a considerable increase (as high as 4x) in particles per unit time collected occurred with a "low" (0.3 vessel volume/mixture) volume air flow. It should be noted that considering only dimensional terms for a cubical box, the change in volume is on the order of $4 \times 10^{-3} \text{ cm}^3/\text{cm}^3/100^\circ \text{ C}$ (or 1.7 cm^3). The aerosol concentration shown in Table 8 exceeds that expected from thermal change by a very large factor which could be computed only with additional information regarding the temperature change rate, which is not expected to be of the order of magnitude to make the pumping action equivalent to that tested here.

CONCLUSIONS

Spores deposited on interior surfaces of a stainless steel container from aerosol, swab or finger imprinting are released at low levels by minute vibrations, thermal cycling and sharp shock. Release fractions appear to be on the order of 0.6 to $3.0 \times 10^{-2}\%$ of original deposits. The released particles are small ($< 3 \mu\text{m}$) and are readily transportable. The majority of release occurs in the first cycle of temperature change, physical shock or even handling. Thereafter, the release rate diminished slowly.

Table 1. Spore burden, inner surface of test vessel

Contamination by	# of trials	Spores/vessel ($\times 10^7$)
Aerosol deposition	17	8.0 ($\sigma = 9.0$)
Cotton Swab	1	3.4
Finger	1	27

Table 2. Effect of varied air flow within test vessel on spore release (Vertical position)

trial	air flow l/min	time, min	*spores/test plate	**CFU/plate radially located plates	total
1	0	5	43	5,3,0	8
	1	5	28	2,9,3	14
	5	5	10	4,2,4	10
	10	5	1000	1,2	3
	85	5	1000+	7,15,6	28
2	0	5	11	3,6,4	13
	1	5	Contam.	3,8,6	17
	5	5	25	11,5,7	23
	10	5	10	5,9,3	17
	85	5	Ca 3000	12,12,19	43

* Directly below unit
TSA filled plates

**10 in. away from base of unit

$$1 = 2.96 \times 10^7$$

Residual BG spores/unit

$$2 = 1.83 \times 10^7$$

$$2 = 300 \text{ ppm w/v}$$

Table 3. Effect of 150 ml/min air flow for 20 hrs. on release of BG spores.

temp. °C	water vapor ppm	time hr.	air flow ml/min.	*Test plate	** radially located plates	total
24	125	1	0	42	0,7,3,2,0,1,0	54
		20	150	2	3,0,1,1,10,1,27;5,3,17	70

* immediately below unit

** 10 in. away from base of unit

Table 4. Effect of 2 hr hold period at 100 C on dislodgement of BG spores

test plate CFU	Radially located plates CFU	total
11	3,4,34,26,19,6,3,1,3,7,15,19,8	159

270 ppm w/v watervapor

$$\text{Residual BG spores/unit} = 2.55 \times 10^8$$

Table 5. Effect of repeated temperature shifts from 4C to 100C dislodgement of BG spores

	temp. range C	temp.,hr	*CFU/test plate	**CFU/radially located plates	total
pre stress period	24	2	7	1,3,3,8,5,7,5,4	43
stress period	4-100 x 4	2	8	2,1,2,3,4,0,1,1,1,2	26

* Directly below unit

** 10 in. from base of unit

275 ppm watervapor

Residual BG/unit = 4.2×10^7

Table 6. Effect of thermal shift from 5 to 95 C on release of BG spores

temp. °C	sample time min.	*CFU/ test plate	**CFU/radially located plates	total CFU
24	15	4	7,0,0,1	12
95	15	30	0,7,5,8	50
5	15	54	11,2,1,0	68

* Directly below unit

** 10 in. from unit base

180 ppm watervapor

BG spores/unit = 2.2×10^7

Table 7. Recovery of BG spores, allowing 2 hr settling time following a rapid temp. shift

temp. range C	time/hr	*CFU/plate	**CFU/radially located plate	total
24° (steady)	2	2	0-4,2,1,8,1,0	16
24 to 100 to 2 to 24	2	62	1,2,4,1,6,4,5,3,1,2	29

* Directly below unit

** 10 in. away from base of unit

150 ppm water vapor

Residual BG/unit 2.27×10^7

Table 8. BG release from horizontal positioned test vessel under thermal stress and low air flow. (.3 Vpm)(1)

Trial	Residual BG/unit	thermal stress period (sequential)	temp. gradient C	BG Collector		Fraction Collected		BG/ltr. of Effluent Aerosol
				Metal Disc	Glass Filter	Metal Disc	Glass Filter	
1	3.03×10^7	1	-47 to +57	4.74×10^3	7.17×10^3	1.56×10^{-4}	3.86×10^{-5}	2.21×10^2
		2	-55 to +55	1.27×10^2	3.07×10^2	4.19×10^{-6}	1.94×10^{-5}	2.40×10^1
		3	-59 to +57	8.00×10^1	3.33×10^1	2.64×10^{-6}	1.10×10^{-6}	6.28×10^0
2	3.61×10^7	1	-57 to +57	1.26×10^3	1.48×10^3	3.49×10^{-5}	4.10×10^{-5}	3.55×10^2
		2	-61 to +59	2.40×10^2	1.83×10^2	6.65×10^{-6}	5.07×10^{-6}	4.39×10^1
		3	-61 to +60	7.76×10^2	1.30×10^2	2.15×10^{-5}	3.60×10^{-6}	3.12×10^1

Sampled during entire stress period (40 min)

(1) Volumes of Vessel/minute

Table 9. Effect of deceleration at 8-12G of test vessel on release of spores.

Trial	Residual BG/unit ($\times 10^7$)	BG Collected per disc ($\times 10^3$)	Fraction released ($\times 10^{-4}$)
1	31.3	88.4	2.82
2	4.59	2.80	0.61
3	22.8	26.6	1.17
4	10.7	17.0	1.59

125 ppm water vapor

Temp. 22 C

Settling time 1 hr.

Table 10. BG release due to 12" drop to hard surface. (G 100)

Test Number	Fall Dist.	Temp.	RH ppm w v	Residual BG/unit	BG recovered /Disc	Initial BG/unit	% Recovered
1	12"	22	80	1.06×10^7	4.32×10^5	1.10×10^7	3.93
2	12"	22	80	5.36×10^7	1.70×10^6	5.33×10^7	3.07
3	12"	22	80	9.10×10^7	2.34×10^6	9.33×10^7	2.51

Table 11. Release of spores from test vessel by thermal cycling.
Contamination by fingers on cotton swab.

Deposition	Temperature °C	Spores Collected	Fraction released ($\times 10^{-5}$)
Cotton swab	-54 to +57	7.3×10^2	2.2
Fingerprint	-57 to +57	4.1×10^4	15.4

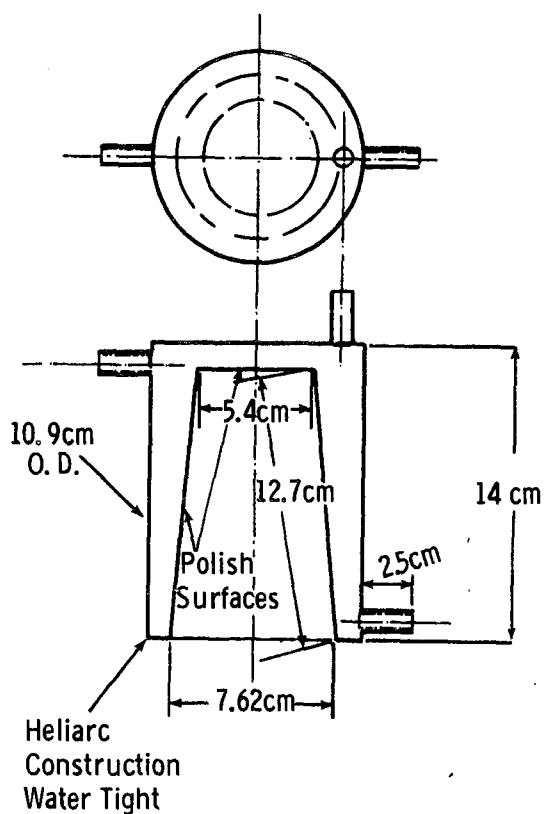


Fig. 1. Fallout test vessel; cross section and plan.

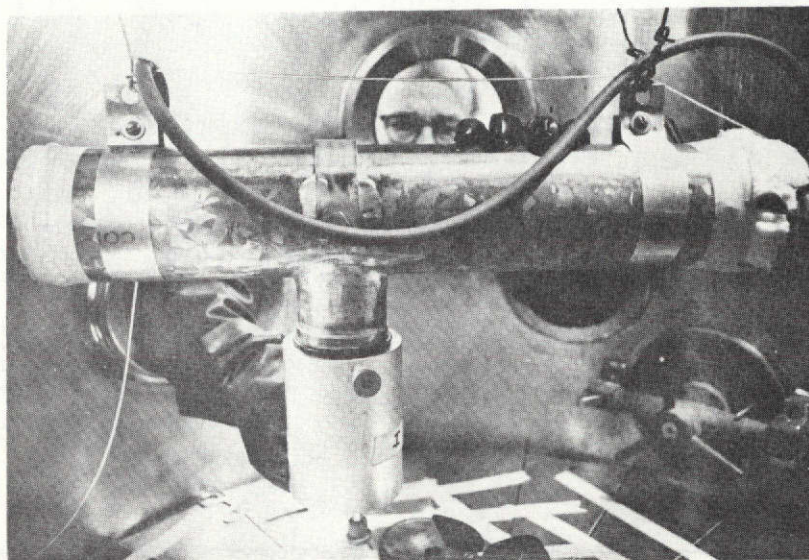


Fig. 2. Aerosol transit tube, shown inside the large tank. The test vessel is shown attached to the T-section. Aerosol generator is on the right hand end of the transit tube.

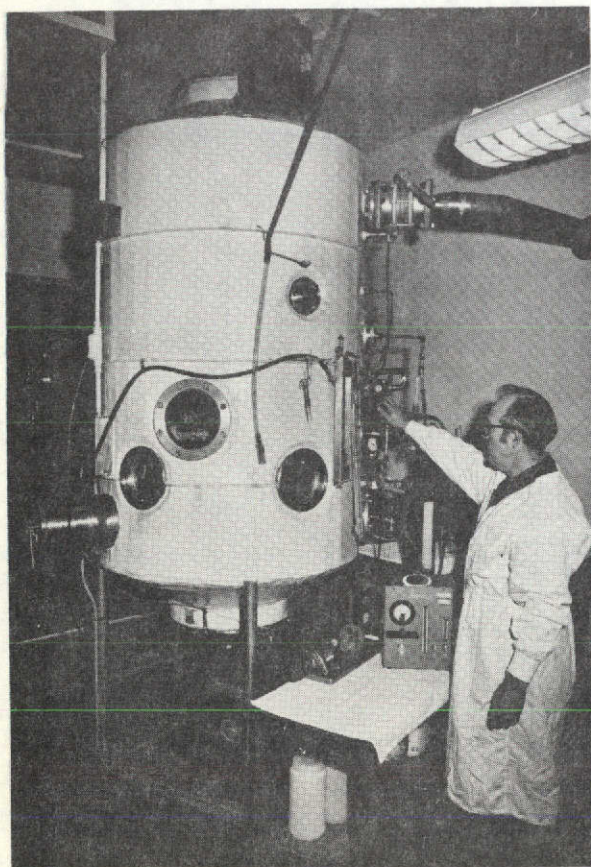


Fig. 3.
Exterior view of large tank.
Transit tube may be seen through
the central view port. The
hygrometer and motorized syringe
are on the towel-covered table.

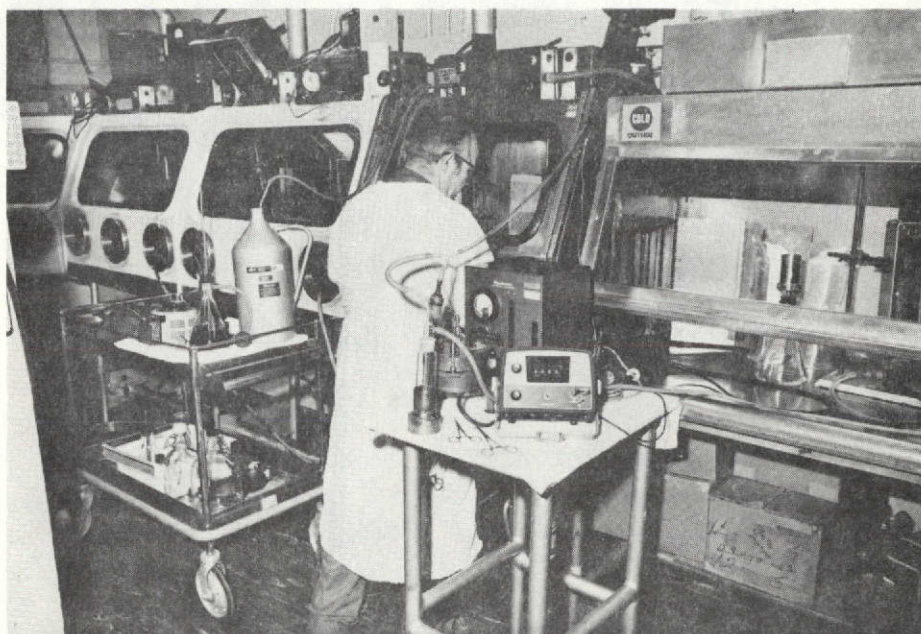


Fig. 4. Test chamber, just to left of open-faced hood. Various test equipment is on cart (liquid N_2 container and steam generator) and on table (hygrometer, impinger, slit sampler and millivoltmeter).

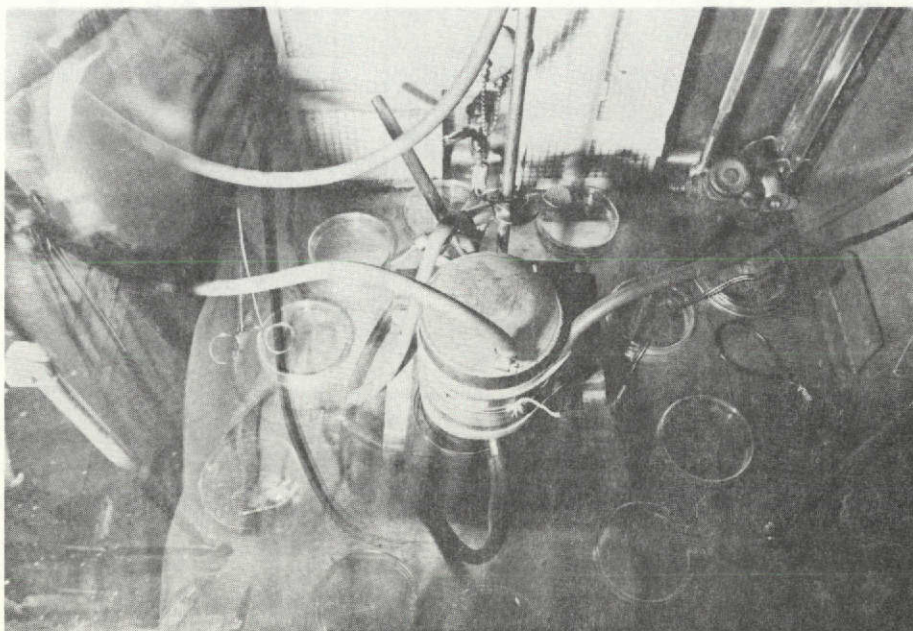


Fig. 5. View inside test chamber. Test vessel is in center, open end down. Petri plates are on a circle below and to the side of the test vessel. One petri dish is directly below. Thermocouple is seen attached to vessel side.

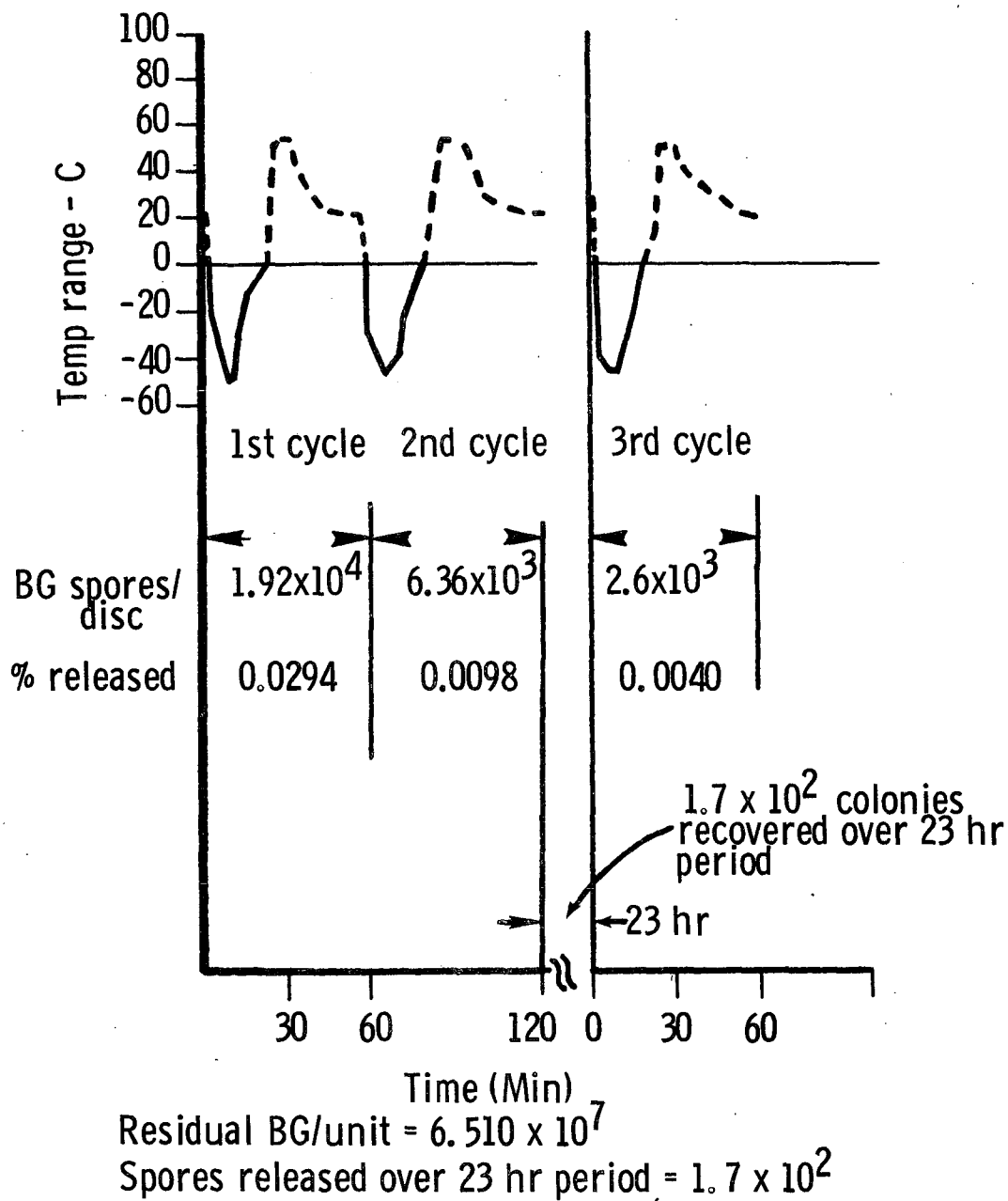


Fig. 6. Quantitative BG release on metal disc over three thermal stress periods.

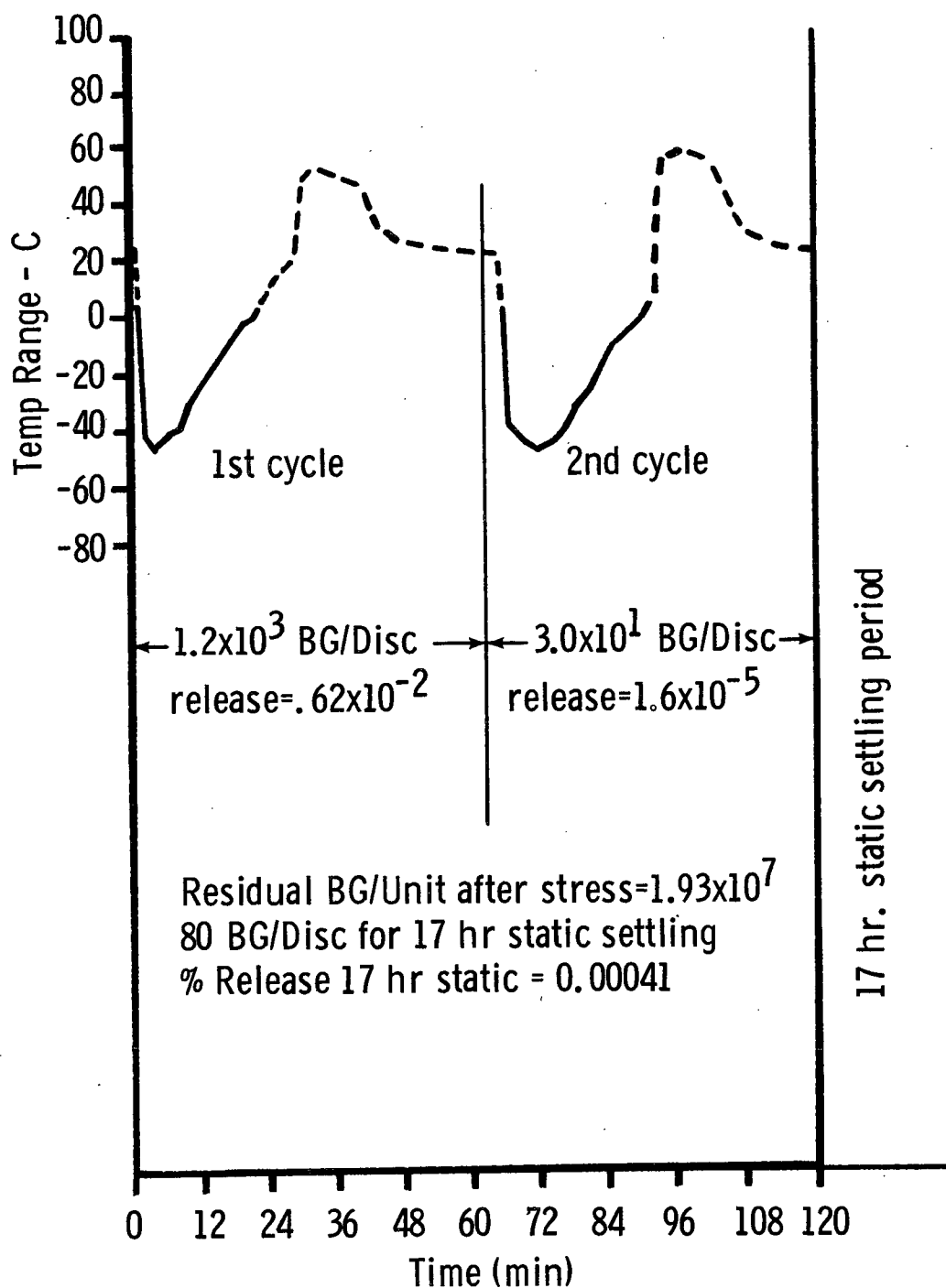


Fig. 7. BG release onto metal disc over two thermal stress periods. Test vessel in horizontal position - no air flow.

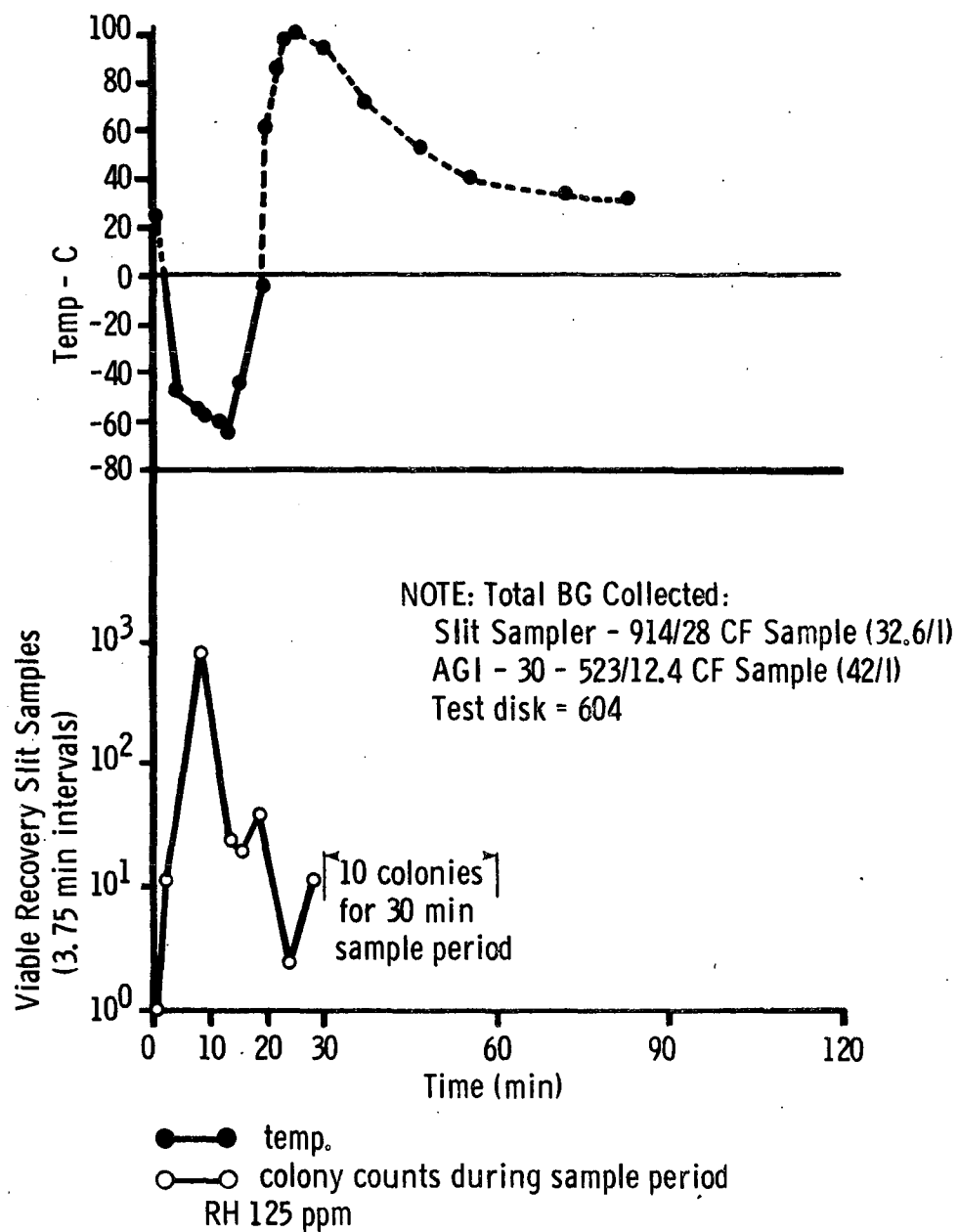


Fig. 8. BG collection in relation to time during one cooling and heating cycle of test vessel. Vertical position.

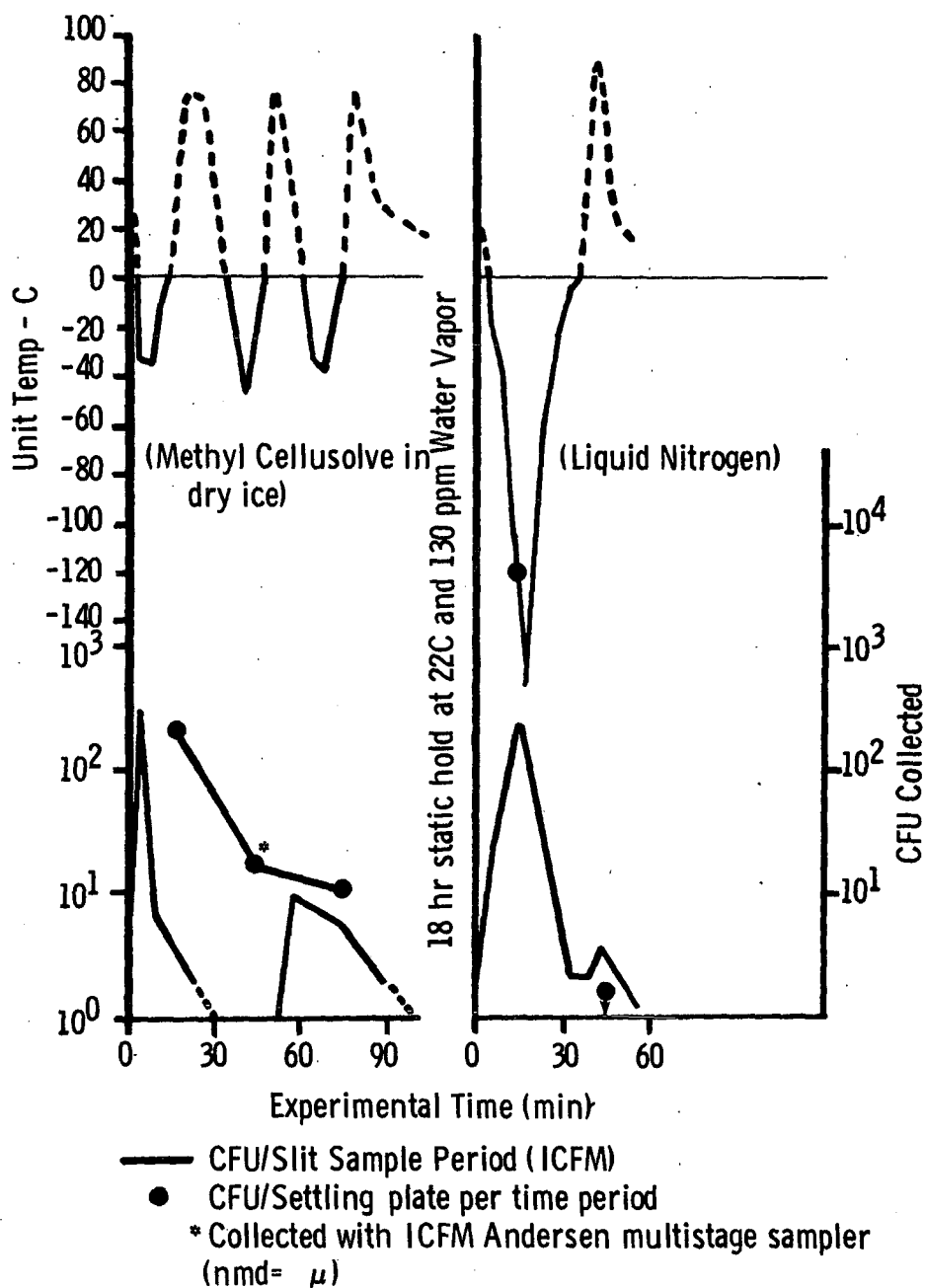


Fig. 9. BG collection in relation to time during cooling and heating cycle.

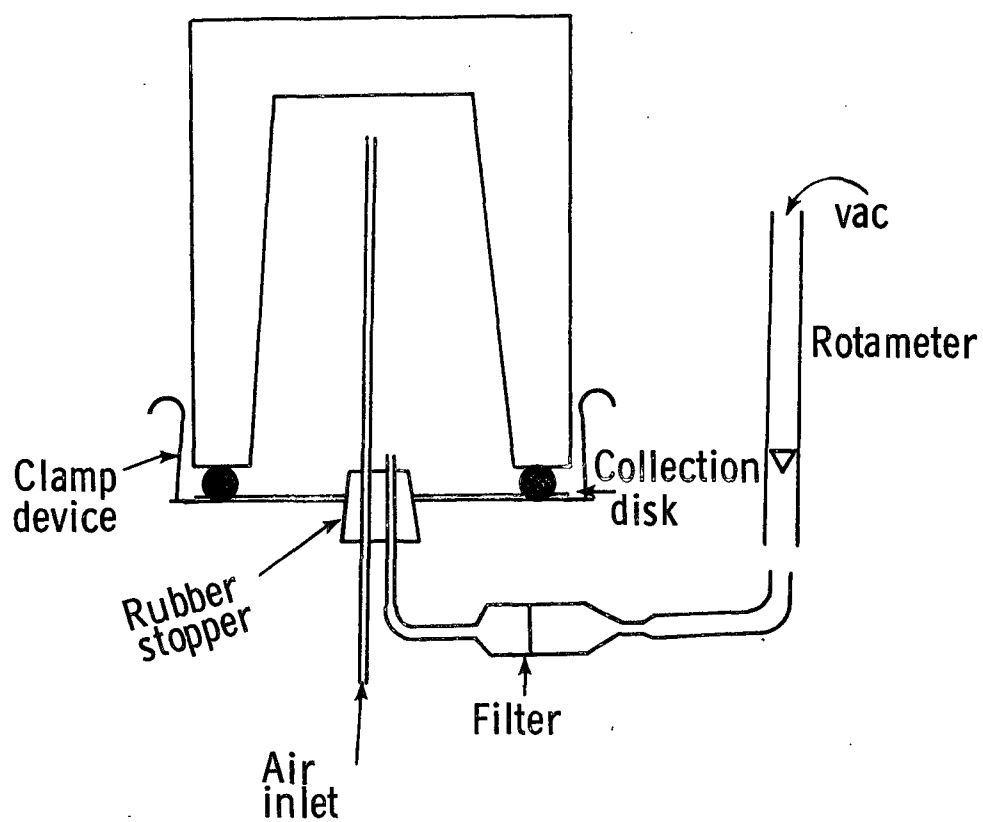


Fig. 10. Schematic of set-up for testing for spore release from test vessel in horizontal position.

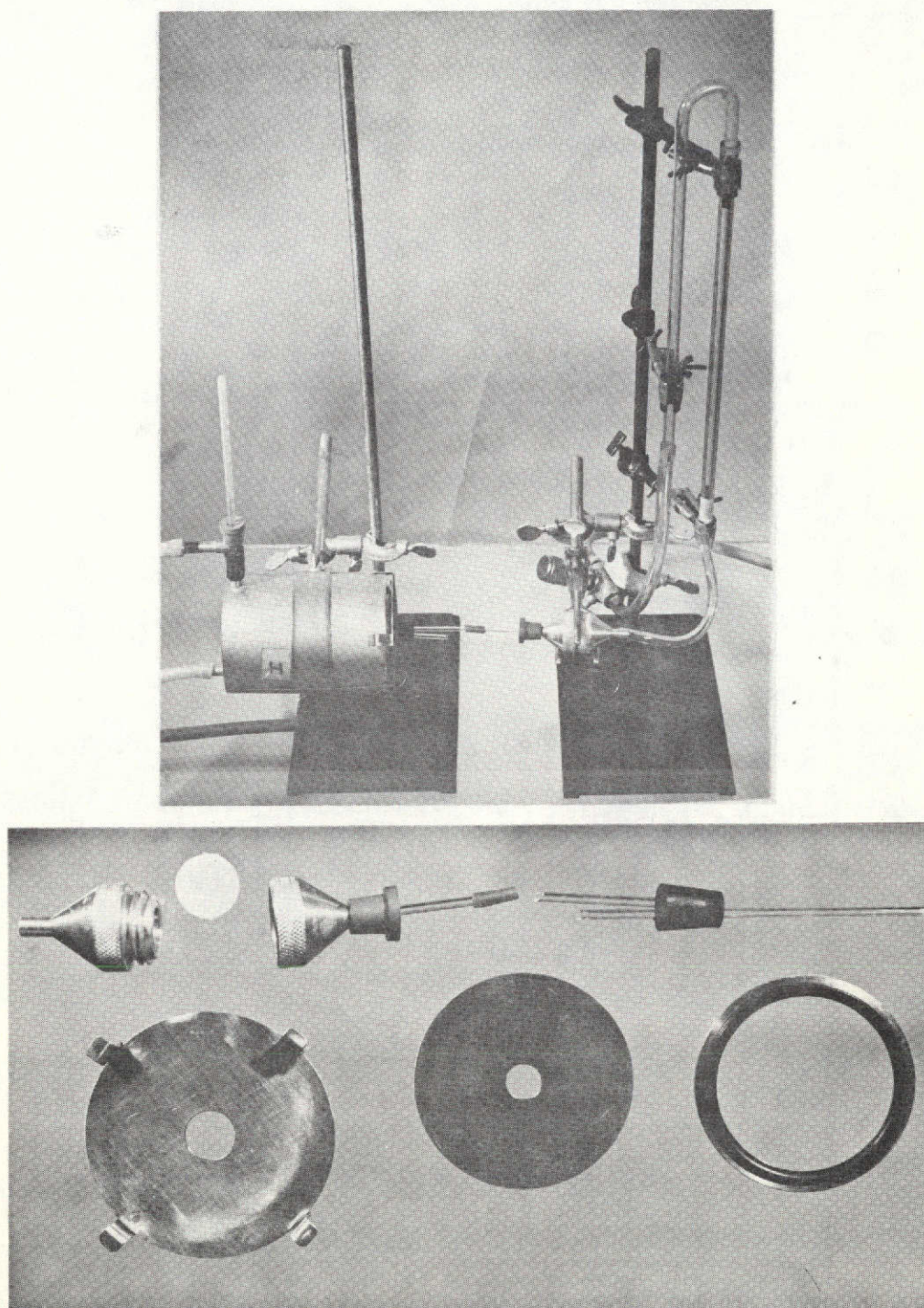


Fig. 11. Test arrangement for study of spore dislodgement with test vessel in horizontal position. Upper-assembled; bottom - filter, collecting disc, support disc and O-ring.

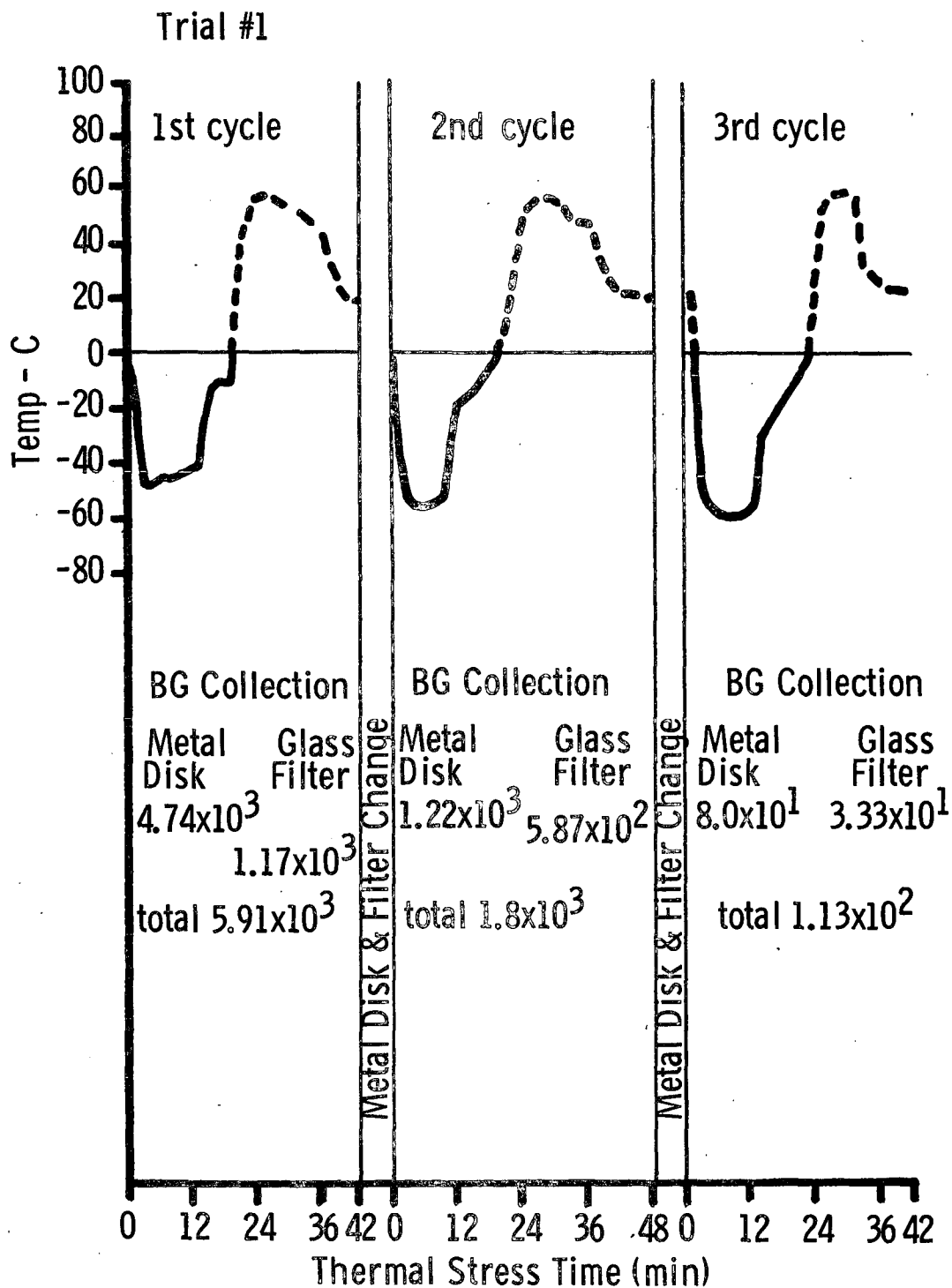


Fig. 12. Collection of spores with test vessel in horizontal position. Temperature sequences.

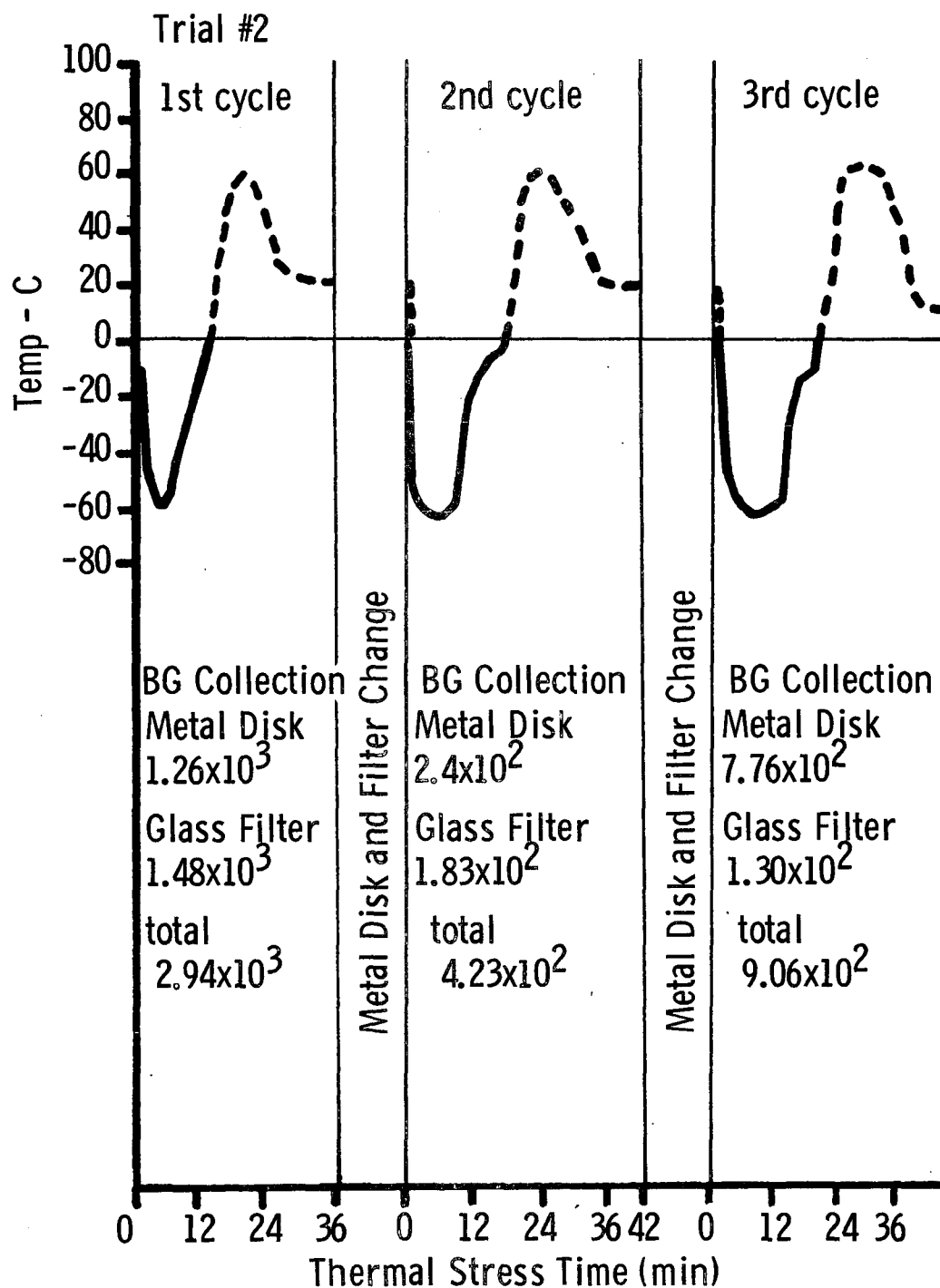


Fig. 13. Collection of spores with test vessel in horizontal position. Temperature sequences.

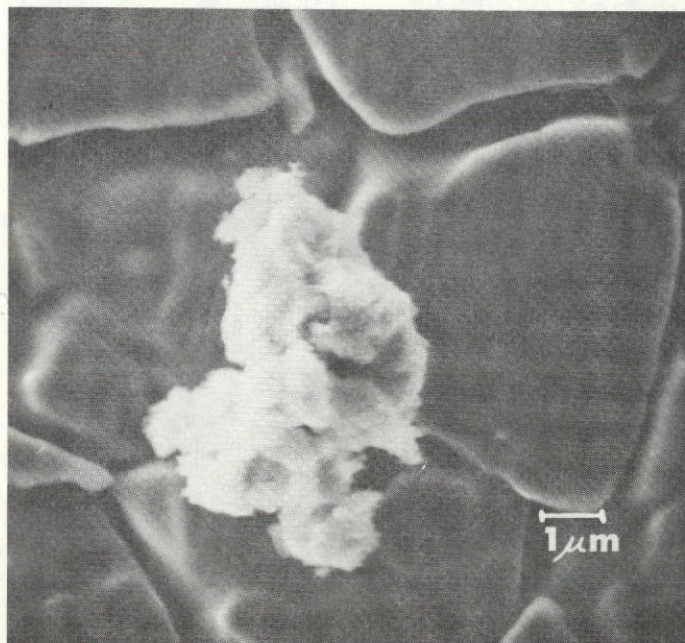


Fig. 14. Spore bearing particle deposited from aerosol onto stainless steel.

ATTENTION REPRO:

BEFORE PRINTING, CONTACT INPUT FOR PAGINATION

PROCESSOR DB

DO NOT PHOTOGRAPH THIS PAGE

CASE FILE